EFFECT OF CLOVE OIL ON PLANT PATHOGENIC BACTERIA
AND BACTERIAL WILT OF TOMATO AND GERANIUM

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SUMMARY

We determined the antibacterial activity of clove oil against seven different genera of plant pathogenic bacteria including Gram-negative *Agrobacterium tumefaciens*, *Erwinia carotovora* pv. *carotovora*, *Pseudomonas syringae* pv. *syringae*, *Ralstonia solanacearum*, and *Xanthomonas campestris* pv. *pelargonii*, as well as Gram-positive *Rhodococcus fascians* and *Streptomyces* spp. Both Gram-positive and Gram-negative bacteria tested were sensitive to clove oil, with *R. solanacearum* being the most sensitive one. Greenhouse experiments were therefore conducted to determine the effect of clove oil as a pre-plant soil fumigant on bacterial wilt of tomato and geranium caused by *R. solanacearum*. Seven days after treating *R. solanacearum*-infested plant growth medium ("soil") with 5 ml of clove oil per kilogram of soil, populations of *R. solanacearum* were reduced to undetectable level, and none of the tomato and geranium plants transplanted into such soil developed wilt symptoms or harbored the bacterium. Our results suggest that clove oil has the potential to be an alternative control measure to combat bacterial wilt of tomato and geranium.

Key words: clove oil, *Ralstonia solanacearum*, plant essential oils, southern bacterial wilt, plant-based bactericide, plant pathogenic bacteria.

INTRODUCTION

Bacterial wilt caused by *Ralstonia solanacearum* is a devastating disease that is distributed worldwide in tropical, subtropical and warm temperate regions (Hayward, 1991). This soilborne vascular disease attacks over 450 plant species including ornamentals such as geranium (Daughtrey *et al.*, 1995) and is a major constraint on production of many economically important crops such as tomato, tobacco, potato and banana (Kelman *et al.*, 1994). The bacterium normally enters plants from the soil through wounds in the roots, then multiplies in the xylem vessels and spreads through the plant's vascular system (Vasse *et al.*, 1995; Wallis and Truter, 1978). Susceptible plants respond to the high bacterial populations by wilting and dying. The bacterium overwinters in diseased plants, plant debris or in the soil. The inoculum can be spread in soil, water or infected propagation materials (Agrios, 1988; Elphinstone, 1996; Janse *et al.*, 2005; Williamson *et al.*, 2002). Control of bacterial wilt depends mainly on the use of resistant cultivars, when available, proper rotation or fallow, and sanitation (Hayward, 1991; USDA, 2004). Such control, however, has been limited due to the pathogen's wide host range, broad distribution, great variability and ability to survive in soil and water (Javier, 1994). Recently, effort has been made to evaluate thymol, a plant essential oil, for its effectiveness as a biofumigant to control *R. solanaceraum* in tomato under field-inoculated conditions (Ji *et al.*, 2005). Application of thymol significantly reduced disease incidence and increased tomato yield (Ji *et al.*, 2005). In addition, phosphorous acid (H_3PO_3) has been found to inhibit *in vitro* growth of *R. solanacearum* and was effective in protecting geranium plants from infection by either race 1 or 3 of the bacterium when applied as a soil drench in greenhouse experiments (Norman *et al.*, 2006).

Clove oil is an essential oil derived from flower-buds, leaves or twigs of the clove plant *Eugenia aromatica* [synonyms *E. caryophyllata* Thunb., *E. caryophyllus* (Sprengel) Bullock et Harr., and *Syzygium aromaticum* (L.) Merrill et Perry] (Lawless, 1995). This spice plant grows in tropical to sub-tropical regions, and is widely cultivated in Indonesia, Madagascar, Sri Lanka, Tanzania and Brazil, with the first two being the main oil-producing countries (Lawless, 1995). Clove oil has been found to be inhibitory to fungi (Thompson and Cannon, 1986), nematodes (Sangwan *et al.*, 1990; Walker and Melin, 1996), human and animal pathogenic bacteria, and one plant pathogenic bacterium, *Erwinia carotovora* (Deans and Ritchie, 1987; Dorman and Deans, 2000; Friedman *et al.*, 2002; Rhayour *et al.*, 2003). It is also effective against insects such as cowpea weevil, which is a pest of storage

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legume (Gunathilagaraj and Kumaraswamy, 1978), in reducing populations of soilborne plant pathogenic fungal pathogens, and in suppressing Fusarium wilt development (Bowers and Locke, 2000, 2004). The effectiveness of clove oil against different genera of plant pathogenic bacteria and plant disease caused by bacteria is largely unknown. In 1941, Ark reported the effectiveness of clove oil in combination with methanol and glacial acid in eradicating crown galls of almond trees (Ark, 1941). In 1963, Maruzzella *et al.* tested *in vitro* activities of 123 essential oils against four phytopathogenic bacteria: *E. carotovora*, *Corynebacterium michiganense*, *Pseudomonas striafaciens* and *P. glycines,* and found clove oils highly inhibitory against all tested bacteria (Maruzzella *et al.*, 1963). The objective of this study was to evaluate the antimicrobial activities of clove oil against seven major groups of plant pathogenic bacteria, as well as its effect in controlling bacterial wilt of tomato and geranium caused by *R. solanacearum*.

MATERIALS AND METHODS

Bacterial strains and growth. Plant pathogenic bacterial strains used in this study, the disease each causes, and their sources are listed in Table 1. All bacterial strains used for growth inhibition study were grown on nutrient agar (NA). To prepare *R. solanacearum* inoculum for plant assays, the bacterium was freshly streaked from a water stock onto triphenyltetrazolium chloride plates (Kelman, 1954), then a single colony was picked and grown overnight in casamino acid peptone glucose broth (Hendrick and Sequeira, 1984) at 28°C with shaking. Appropriate concentrations of the bacterial suspensions were made in sterile water using OD_{600} as an initial measurement of cell density. Final inoculum cell density was confirmed by dilution plating. To detect *R. solanacearum* in plant stem and soil, tissue homogenates or soil samples were dilution plated onto modified semiselective medium South Africa (SMSA) agar plates (Elphinstone *et al.*, 1996) from which crystal violet was omitted for a better viewing of colony colors.

***In vitro* effect of clove oil on bacterial growth by agar diffusion technique.** Ten milliliters of NA were added to separate sterile Petri plates (60 x 15 mm) and allowed to set. Fifty microliters of each bacterial culture (OD_{600} of 0.2) were then pipetted and evenly spread onto each NA plate in three replicate plates (approximately 10^7 cells per plate). This was followed by removing a well of 5 mm in diameter in the center of each agar plate using a sterilized cork borer. Either 10 or 50 µl of clove oil (Sigma Aldrich, USA) was added into each well. Sterile water was used as a negative control for growth inhibition. The plates were sealed with parafilm and left undisturbed to allow diffusion of the oil sample

into the agar, and incubated in the dark at 28°C for two days, then at room temperature for three more days until zones of growth inhibition (diameter, cm) including the 0.5 cm of the center well diameter were measured and recorded. The experiment was repeated three times.

Soil infestation and treatment. "Pro-mix" plant growth medium (Premier Horticulture, USA) was used as "soil" in our experiments. For each soil experiment, two kilograms of the dry soil was thoroughly mixed with 200 ml of *R. solanacearum* inoculum [10^9 colony-forming units (CFU/ml)] in a plastic bag. Then, the infested soil was treated with 200 ml of 5% (v/v) of clove oil (Sigma Aldrich, USA) emulsified in a carrier solution containing 0.5% (w/v) L-α-lecithin (Sigma Aldrich, USA), 0.1% (v/v) Triton X114 (Rohm and Haas, USA) and sterile water to obtain a final concentration of 0.5% of clove oil (0.5 ml of clove oil per 100 gram of soil). The infested soil treated with only the carrier solution served as an untreated control. After the treatment, the soil was mixed thoroughly and incubated in a closed bag in the dark at room temperature for four days for fumigation to occur. Following the incubation, the bag was opened to allow as much clove oil to dissipate as possible. This was done by moistening the surface soil with 100 ml sterile water every other day, and stirring the soil once every day for seven days. Finally, the soil from each treatment was distributed to 5-inch pots into which six-week-old geranium (*Pelargonium* x *hortorum*) (Goldsmith Seeds, USA) or two to three-week-old tomato (*Lycopersicon esculentum* Mill. cv. Bonny Best) seedlings were transplanted, respectively. Plants were color coded and plant location was randomized across treatment. All plants were kept in a greenhouse between 24°C (night) to 30°C (day) with 14 h of light. There were 10 plants per treatment in each experiment, and the experiment was repeated three times.

Sampling of soil and plant stems. To determine bacterial populations in treated soils at the time of transplanting, one gram of soil was taken from each of the different soil treatment bags containing *R. solanacearum*. The soil sample was placed into 10 ml of sterile water contained in a 50 ml sterile disposable centrifuge tube, shaken at 225 rpm at room temperature for 30 min, followed by dilution plating on the modified SMSA plates. Plates were incubated at 28°C until colonies were apparent. Randomly selected colonies from colonies typical of *R. solanacearum* were tested by PCR using *R. solanacearum*-specific primers (Ito *et al.*, 1998) to confirm their identity.

To detect whether symptomless plants growing in *R. solanacearum*-containing soil treatments were latently infected by the bacterium, a 3 cm stem segment spanning the crown from each of the asymptomatic plants

was sterilized in 10% household bleach for 1 min, followed by two 1 min washes in sterile water. A 1 cm internal segment was then excised from the 3 cm stem piece and ground in 500 µl sterile water in a 1.7 ml microcentrifuge tube (Huang and Allen, 2000). The homogenates were dilution plated on the modified SMSA plates.

Assessment for bacterial wilt and plant growth. The number of wilted plants in each soil treatment was recorded two times a week. Other abnormal symptoms on leaves were also monitored and recorded.

At the end of each experiment, all surviving plants were cut at the soil line. The height and weight of the tomato and geranium plants above the soil line, as well as the weight of their roots were measured and recorded. Roots were washed thoroughly to remove any soil residue and blotted dry with paper towels before measurements. The number of flowering stalks in geranium plants was also recorded.

Statistical analysis. Data on disease severity and plant growth parameters were analyzed by ANOVA using a web-based statistical software at http://faculty.vassar.edu/lowry/anova1u.html. Means were compared using the Tukey's Honestly Significantly Different test provided by the software.

RESULTS

Effect of clove oil on growth of plant pathogenic bacteria. To find out whether clove oil is inhibitory to plant pathogenic bacteria, seven bacteria representing seven genera of plant pathogenic bacteria were chosen for our inhibition study using agar diffusion technique. The bacteria tested included five Gram-negative bacteria, *Agrobacterium tumefaciens*, *Erwinia carotovora* pv. *carotovora*, *Pseudomonas syringae* pv. *syringae*, *Ralstonia solanacearum*, and *Xanthomonas campestris* pv. *pelargonii*, as well as two Gram-positive bacteria, *Rhodococcus fascians* and *Streptomyces* spp. The growth of both Gram-positive and Gram-negative bacteria was significantly inhibited by clove oil at both the volumes tested, as compared to their individual water controls (Table 2). The degree of sensitivity, however, varied among different bacterial species and the amount of clove oil used (Table 2). When 50 µl of clove oil was used in our agar diffusion assay, *R. solanacearum* was the most sensitive, followed by *X. campestris* pv. *campestris*, *R. fascians*, *Streptomyces* spp., *P. syringae* pv. *syringae*, *A. tumefaciens* and *E. carovotora* (Table 2). A full lawn of bacterial growth was observed on all the water control plates.

Effect of clove oil on population of *R. solanacearum* in soil, bacterial wilt, and growth of tomato and gerani-

Table 1. Bacterial strains used in this study.

Bacterial strain	Disease	Reference or source
Agrobacterium tumefaciens strain EHA 105	Crown gall	Le *et al.* (2001)
Erwinia carotovora pv. *carotovora* strain 71	Soft rot of vegetables	McEvoy *et al.* (1990)
Pseudomonas syringae pv. *syringae*	Brown spot of bean	Atkinson and Baker (1987)
Ralstonia solanacearum strain K60 (race 1, biovar 1)	Bacterial wilt of tomato and geranium	Kelman (1954)
Rhodococcus fascians strain 76	Leafy gall of veronica	M. Putnam, Oregon State University
Streptomyces spp. isolate ME02-6979-5A	Potato scab	L. Wanner, USDA-ARS
Xanthomonas campestris pv. *pelargonii*	Bacterial blight of geranium	J. S. Hartung, USDA-ARS

Table 2. Zones of growth inhibition (cm in diameter) showing antibacterial activity of clove oil.

Bacterial strain	Volume (µl) of clove oil in center well		
	0	10	50
Agrobacterium tumefaciens strain EHA 105	0 ± 0 a*	1.83 ± 0.22 b	2.37 ± 0.23 b
Erwinia carotovora pv. *carotovora* strain 71	0 ± 0 a	1.53 ± 0.15 b	1.87 ± 0.23 b
Pseudomonas syringae pv. *syringae*	0 ± 0 a	2.50 ± 1.25 b	2.77 ± 1.11 b
Ralstonia solanacearum strain K60 (race 1, biovar 1)	0 ± 0 a	3.67 ± 0.12 b	4.93 ± 0.07 c
Rhodococcus fascians strain 76	0 ± 0 a	2.73 ± 0.68 b	3.30 ± 0.60 b
Streptomyces spp. isolate ME02-6979-5A	0 ± 0 a	2.47 ± 0.12 b	2.90 ± 0.06 c
Xanthomonas campestris pv. *pelargonii*	0 ± 0 a	2.97 ± 0.69 b	3.40 ± 0.60 b

*Values for zone of inhibition (including the 0.5 cm of the center well diameter) are presented as the means of three separate experiments, each containing three plates per bacterium, plus standard error.

Values followed by different letters within each row are significantly different ($p \leq 0.05$) based on Tukey's HSD test.

um plants. Since *R. solanacearum* was found to be most sensitive to clove oil, we conducted greenhouse experiments to test the effect of clove oil as a pre-plant soil fumigant against bacterial wilt of tomato and geranium. In our preliminary study, we tested three concentrations of clove oil, 0.1%, 0.25% and 0.5%. We also tested 0.5% soybean oil to determine the effect of oil per se on control of bacterial disease. Only 0.5% clove oil protected both the tomato and geranium plants from infection by *R. solanacearum*, while 0.1% and 0.25% clove oil had some and 0.5% soybean oil had no significant effect on prevention of bacterial wilt of tomato and geranium (data not shown). We therefore chose 0.5% clove oil for further study.

At the time of transplanting tomato or geranium plants into *R. solanacearum*-infested soil, *R. solanacearum* was not detectable in such soil treated with 0.5% clove oil, while 7.5×10^8 CFU gram^{-1} soil was detected in the infested soil treated with only carriers.

Four weeks after tomato plants were transplanted into soils infested with *R. solanacearum*, none of the tomato plants grown in such soil treated with 0.5% clove oil developed wilt symptoms and no latent infection was detected in any of the asymptomatic plants (Table 3). For geranium, six weeks after transplanting into *R. solanacearum*-infested soils, all the plants grown in such soil treated with 0.5% clove oil were free of wilt symptoms and the bacterium (Table 4). All tomato and geranium plants grown in *R. solanacearum*-infested soil without clove oil treatment (carrier control treatment) developed wilt symptoms and had significantly higher disease severity (Tables 3 and 4).

In *R. solanacearum*-free soils, tomato and geranium plants in 0.5% clove oil treatment had similar aboveground height and weight and root weight, as compared to their untreated controls and those grown in bacterium infested soil treated with clove oil (Tables 3 and 4). Both tomato and geranium plants grown in *R.*

Table 3. Effect of clove oils on development of bacterial wilt and growth of tomato plants.

Treatment		Plants with wilt symptoms[b]	Plants with Rs[c]	Above ground plant height (cm)[d]	Above ground plant weight (g)[d]	Plant root weight (g)[d]
0.5% Clove oil[a]	- Rs	0 ± 0	ND	53.11 y	39.83 y	16.58 y
	+ Rs	0 ± 0	0 ± 0	54.88 y	43.07 y	16.50 y
Untreated control	- Rs	0 ± 0	ND	52.04 y	43.68 y	16.92 y
	+ Rs	10 ± 0	10 ± 0	12.45 z	5.68 z	4.28 z

[a]Five milliliters of clove oil per one kilogram of soil were applied to soil after the soil was infested with *R. solanacearum* (+ Rs) or untreated (- Rs). Tomato plants were transplanted after the soil was fumigated for 4 days followed by 7 days of aeration.
[b,c]Values are the means of three separate experiments, each containing 10 plants, plus standard error.
[c]*R. solanacearum* was isolated at the end of each experiment from basal stem tissue of all plants grown in soil containing *R. solanacearum*.
[d]Values are the means of three separate experiments, each containing 10 plants. Values followed by different letters within each column are significantly different ($p \leq 0.01$) based on Tukey's HSD test.
ND: not determined.

Table 4. Effect of clove oil on development of bacterial wilt and growth of geranium plants.

Treatment		Plants with wilt symptoms[b]	Plants with Rs[c]	Above ground plant height (cm)[d]	Above ground plant weight (g)[d]	Plant root weight (g)[d]	No. of flowering stalks[d]
0.5% Clove oil[a]	- Rs	0 ± 0	ND	24.27 y	34.37 y	12.41 y	5.9 y
	+ Rs	0 ± 0	0 ± 0	20.48 y	29.82 y	10.59 y	4.5 y
Untreated control	- Rs	0 ± 0	ND	22.33 y	32.82 y	10.39 y	4.7 y
	+ Rs	10 ± 0	10 ± 0	3.33 z	2.33 z	0.31 z	0.9 z

[a]Five milliliters of clove oil per one kilogram of soil were applied to soil after the soil was infested with *R. solanacearum* (+ Rs) or untreated (- Rs). Geranium plants were transplanted after the soil was fumigated for 4 days followed by 7 days of aeration.
[b,c]All values are the means of three separate experiments, each containing 10 plants, plus standard error.
[c]*R. solanacearum* was isolated at the end of each experiment from basal stem tissue of all plants grown in soil containing *R. solanacearum*.
[d]Values are the means of three separate experiments, each containing 10 plants. Values followed by different letters within each column are significantly different ($p \leq 0.01$) based on Tukey's HSD test.
ND: not determined.

solanacearum-infested soils pre-treated with 0.5% clove oil were significantly taller and produced significantly more root and above-ground plant tissues than their untreated controls (Tables 3 and 4). Similarly, geranium plants grown in *R. solanacearum*-infested soils treated with 0.5% clove oil produced similar number of flowering stalks to those in bacterium-free soil with or without clove oil treatment. Those plants, however, bore significantly more flowering stalks than plants grown in *R. solanacearum*-infested soils without clove oil treatment (Table 4).

DISCUSSION

Clove oil is volatile and generally recognized as safe for use in dental cement or as food additives. Its antimicrobial effect has mostly been studied *in vitro* for animal and human bacteria (Deans and Richie, 1987; Dorman and Deans, 2000), or food spoilage microorganisms and food-poisoning bacteria (Deans and Richie, 1987). In agriculture, its *in vitro* effect on plant pathogens and potential use against plant diseases have rarely been explored (Ark, 1941; Bowers and Locke, 2000, 2004; Dorman and Dean, 2000; Maruzzella *et al*., 1963). Our study is the first to examine the effect of clove oil on plant pathogenic bacteria from seven different genera and its potential as an alternative control measure to combat bacterial wilt of tomato and geranium.

All the bacteria tested in our study, including both Gram-positive and Gram-negative ones, were sensitive to clove oil, although with various degrees at certain clove oil concentrations. The two Gram-positive bacteria tested were not more resistant than all of the Gram-negative ones, consistent with the results of Deans and Richie (1987) that the Gram reaction appears to have little correlation to growth inhibition of bacteria by plant volatile oils. Among the bacteria tested, *R. solanacearum* was the most sensitive as shown by the agar diffusion test. Greenhouse experiments were therefore conducted to test the effect of clove oil as a pre-plant soil fumigant to control bacterial wilt of tomato and geranium caused by *R. solanacearum*. When *R. solanacearum*-infested soil was treated with clove oil at 0.5% or 5 ml per kilogram of soil, no *R. solanacearum* could be recovered from the soil and no plants grown in such soil developed wilt symptoms or harbored *R. solanacearum*, suggesting that at this concentration and under our experimental condition, clove oil is effective to eliminate *R. solanacearum* in the soil and to protect tomato and geranium plants from infection by the bacterium.

Pradhanang *et al.* (2003) reported that some tomato plants grown in soil treated with palmarosa oil developed chlorotic and necrotic leaf margins, although the new growth of these plants did not seem to be affected.

Similar observations were also made by us in our preliminary experiments when tomato and geranium plants were grown in soils fumigated with clove oil for four days followed by three days of aeration (data not shown). Chlorotic and necrotic symptoms on lower leaves of tomato and geranium plants, however, were not observed in this study when the soil was allowed to aerate for seven instead of three days to vent excess clove oil. This suggests that the longer the aeration period, the less phytotoxicity effect clove oil may have on the plants.

Clove oil treatment did not seem to reduce, if not promote, the growth of some of the other bacteria in the soil as evidenced by the background contaminating bacteria on the modified semi-selective SMSA plates (data not shown), making quantifying *R. solanacearum* in such soil complicated. A similar observation was also reported by Pradhanang *et al.* (2003) in *R. solanacearum*-infested soils treated with thymol, palmarosa, or lemongrass oils.

In order to determine whether the antibacterial effect of clove oil was caused by oil per se, we tested 0.5% soybean oil in our preliminary experiments. Although soybean oil reduced population of *R. solanacearum* in infested soil by about 100 fold, the surviving populations of the bacterium were enough to cause similar levels of disease severity, wilted similar numbers of plants and caused latent infection, as compared with untreated controls (data not shown). This suggests that components of clove oil are the major contributors to its antibacterial properties.

Our results demonstrated that clove oil has the potential to eliminate populations of *R. solanacearum* in infested soils. In addition, depending on its concentrations used, clove oil could either protect tomato and geranium plants from infection by the bacterium or greatly reduce bacterial wilt incidence under greenhouse conditions. The effectiveness of clove oil to control bacterial wilt and the economics of its application under field conditions, however, remain to be determined. Ji *et al.* (2005) have evaluated the plant essential oil thymol under field inoculated conditions, and found its application significantly reduced bacterial wilt incidence and increased plant yield of a susceptible tomato cultivar. Since clove oil is a mixture of different compounds including mainly eugenol, as well as eugenyl acetate, caryophyllene and other minor constituents (The Merck Index, 2001), how these components contribute to its antibacterial properties also remains to be studied.

As shown by this and previous studies, clove oil and other plant essential oils possess a broad-spectrum activity against a wide range of plant pathogens including bacteria, fungi and nematodes in soil (Bowers and Locke, 2000, 2004; Momol *et al.*, 2000; Oka *et al.*, 2000). These plant essential oils, therefore, have the potential to be developed into an environmentally friendly and sound alternative to the use of methyl bromide in integrated management of soil-borne plant diseases.

REFERENCES

Agrios G.N., 1988. Plant diseases caused by prokaryotes. In: Agrios G.N. (ed.). Plant Pathology, pp. 510-610. Academic Press, New York, NY, USA.

Ark P.A., 1941. Chemical eradication of crown gall on almond trees. *Phytopathology* **31**: 956-957.

Atkinson M.M., Baker C.J., 1987. Alteration of plasmalemma sucrose transport in *Phaseolus vulgaris* by *Pseudomonas syringae* pv. *syringae* and its association with K+/H+ exchange. *Phytopathology* **77**:1573-1578.

Bowers J.H., Locke J.C., 2000. Effect of botanical extracts on the population density of *Fusarium oxysporum* in soil and control of Fusarium wilt in the greenhouse. *Plant Disease* **84**: 300-305.

Bowers J.H., Locke J.C., 2004. Effects of formulated plant extracts and oils on population density of *Phytophthora nicotianae* in soil and control of Phytophthora blight in the greenhouse. *Plant Disease* **88**: 11-16.

Daughtrey M.L., Wick R.L., Peterson J.L., 1995 Compendium of Fowering Potted Plant Diseases, pp. 52-53. APS Press, St. Paul, MN, USA.

Deans S.G., Ritchie G., 1987. Antibacterial properties of plant essential oils. *International Journal of Food Microbiology* **5**: 165-180.

Dorman H.J.D., Deans S.G., 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology* **88**: 308-316.

Elphinstone J.G., 1996. Survival and possibility for extinction of *Pseudomonas solanacearum* (Smith) Smith in cool climates. *Potato Research* **39**: 403-410.

Elphinstone J.G., Hennessey J., Wilson J.K., Stead D.E., 1996. Sensitivity of different methods for the detection of *Ralstonia solanacearum* in potato tuber extracts. *Bulletin OEPP/EPPO Bulletin* **26**: 663-678.

Friedman M., Henika P.R., Mandrell R.E., 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni, Escherichia coli, Listeria monocytogenes*, and *Salmonella enterica. Journal of Food Protection* **65**: 1545-1560.

Gunathilagaraj K., Kumaraswamy T., 1978. Laboratory evaluation of toxicity of clove oil to *Callosobruchus chinensis* (L.) on greengram seeds. *The Madras Agricultural Journal* **65**: 487-488.

Hayward A.C., 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum. Annual Review of Phytopathology* **29**: 65-87.

Hendrick C., Sequeira L., 1984. Lipopolysaccharide-defective mutants of the wilt pathogen *Pseudomonas solanacearum. Applied and Environmental Microbiology* **48**: 94-101.

Huang Q., Allen C., 2000. Polygalacturonases are required for rapid colonization and full virulence of *Ralstonia solanacearum* on tomato plants. *Physiological and Molecular Plant Pathology* **57**: 77-83.

Ito S., Ushijima Y., Fujii T., Tanaka S., Kameya-Iwaki M., Yoshiwara S., Kishi F., 1998. Detection of viable cells of *Ralstonia solanacearum* in soil using a semiselective medium and a PCR technique. *Journal of Phytopathology* **146**: 379-384.

Janse J.D., Beld H.E.V.D., Elphinstone J., Simpkins S., Tjou-Tam-Sin L.N.A., Vaerenbergh J.V., 2005. Introduction to Europe of *Ralstonia solanacearum* biovar 2, race 3 in *Pelargonium zonale* cuttings. In: Allen C., Prior P., Hayward A.C. (eds). Bacterial Wilt: The Disease and the *Ralstonia solanacearum* Species Complex, pp. 81-94. APS Press, St Paul, MN, USA.

Javier E.Q., 1994. Foreword. In: Hayward A.C., Hartman G.L. (eds). Bacterial Wilt, pp. 11-12. CAB International, Oxon, UK.

Ji P., Momol M.T., Olson S.M., Pradhanang P.M., Jones J.B., 2005. Evaluation of thymol as biofumigant for control of bacterial wilt of tomato under field conditions. *Plant Disease* **89**: 497-500.

Kelman A., 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on tetrazolium medium. *Phytopathology* **44**: 693-695.

Kelman A., Hartman G.L., Hayward A.C., 1994. Introduction. In: Hayward A.C., Hartman G.I. (eds). Bacterial Wilt, pp. 1-7. CAB International, Oxon, UK.

Lawless J., 1995. The illustrated encyclopedia of essential oils: The complete guide to the use of oils in aromatherapy and herbalism. Element Books, London, UK.

Le V.Q., Belles-Isles J., Dusabenyagasani M., Tremblay F.M., 2001. An improved procedure for production of white spruce (*Picea glauca*) transgenic plants using *Agrobacterium tumefaciens. Journal of Experimental Botany* **52**: 2089-2095.

Maruzzella J.C., Reine S., Solat H., Zeitlin A., 1963. The action of essential oils on phytopathogenic bacteria. *Plant Disease Reporter* **47**: 23-26.

McEvoy J.L., Murata H., Chatterjee A.K., 1990. Molecular cloning and characterization of an *Erwinia carotovora* subsp. *carotovora* pectin lyase gene that responds to DNA-damaging agents. *Journal of Bacteriology* **172**: 3284-3289.

Momol M.T., Mitchell D.J., Rayside P.A., Olson S.M., Momol E.A., 2000. Plant essential oils as potential biofumigants for the management of soilborne pathogens of tomato. *Phytopathology* **90**: S127.

Norman D.J., Chen J., Yuen J.M.F., Mangravita-Novo A., Byrne D., Walsh L., 2006. Control of bacterial wilt of geranium with phosphorous acid. *Plant Disease* **90**: 798-802.

Oka Y., Nacar S., Putievsky E., Ravid U., Yaniv Z., Spiegel Y. 2000. Nematicidal activity of essential oils and their components against the root-knot nematode. *Phytopathology* **90**: 710-715.

Pradhanang P.M., Momol M.T., Olson S.M., 2003. Effects of plant essential oils on *Ralstonia solanacearum* population density and bacterial wilt incidence in tomato. *Plant Disease* **87**: 423-427.

Rhayour K., Bouchiki T., Tantaoui-Elaraki A., Sendide K., Remmal A., 2003. The mechanism of bactericidal action of oregano and clove essential oils and their phenolic major components on *Escherchia coli* and *Bacillus subtilis*. *Journal of Essential Oil Research* **15**: 356-362.

Sangwan N.K., Verma B.S., Verma K.K., Dhindsa K.S., 1990. Nematicidal activity of some essential plant oils. *Pesticide Science* **28**: 331-335.

The Merck Index, 2001. 13th Ed. Merck & CO, Whitehouse Station, NJ, USA.

Thompson D.P., Cannon C., 1986. Toxicity of essential oils and toxicogenic and nontoxicogenic fungi. *Bulletin of Environmental Contamination and Toxicology* **36**: 527-532.

United States Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS), 2004. Minimum Sanitation Protocols for Offshore Geranium Cutting Production. USDA-APHIS, Riverdale, MD, USA.

Vasse J., Frey P., Trigalet A., 1995. Microscopic studies of intercellular infection and protoxylem invasion of tomato roots by *Pseudomonas solanacearum*. *Molecular Plant-Microbe Interaction* **8**: 241-251.

Walker J.T., Melin J.B., 1996. *Mentha x piperita*, *Mentha spicata* and effects of their essential oils on Meloidogyne in soil. *Journal of Nematology* **28**: 692-635.

Wallis F.M., Truter S.J., 1978. Histopathology of tomato plants infected with *Pseudomonas solanacearum*, with emphasis on ultrastructure. *Physiological Plant Pathology* **13**: 307-317.

Williamson L., Allen C., Nakaho K., Hudelson B., 2002. *Ralstonia solanacearum* race 3, biovar 2 strains isolated from geranium are pathogenic on potato. *Plant Disease* **86**: 87-991.